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Studies on Antioxidant Properties of *Catharanthus rosea* and *Catharanthus alba*.

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Abstract
Various forms of activated oxygen, generally known as reactive oxygen species (ROS) which also include free radicals are implicated for more than 80 diseases including Diabetes mellitus, arthritis, cancer, ageing etc. In treatment of these diseases, antioxidant therapy has gained utmost importance. Current research is now directed towards finding naturally occurring antioxidant of plant origin. Plant and plant products are being used as a source of medicine since long. In Indian system of medicine Catharanthus roseus is used in Ayurvedic medicines for number of ailments. The present study deals with comparative evaluation of antioxidant potential of ethanolic extracts of flowers of two varieties of Catharanthus roseus L. namely 'rosea'(pink flowers) and 'alba'(white flowers) using different systems of assay, e.g. Hydroxyl radical-scavenging activity, superoxide radical-scavenging activity, DPPH radical- scavenging activity and nitric oxide radical inhibition method. The results revealed that the ethanolic extracts of flowers of Periwinkle varieties extracts exhibited satisfactory scavenging effect in all the radical scavenging assays in a concentration dependent manner; however Catharanthus rosea had more antioxidant activity than Catharanthus alba.

Key Words
Efavir Antioxidant, Catharanthus rosea, Catharanthus alba.

Introduction
Living cells are continuously exposed to a variety of challenges that exert oxidative stress which arises in a biological system after an increased exposure to oxidants, a decrease in the antioxidant capacity of the system, or both[1]. It is often associated with or leads to the generation of reactive oxygen species including free radicals which implicated in the pathophysiology of various diseases. Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function[2]. Reactive oxygen species (ROS) is a term which encompasses all highly reactive, oxygen-containing molecules, including free radicals. Types of ROS include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage[3]. Fortunately; the formation of free radicals is controlled naturally by various beneficial compounds known as antioxidants. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating. As plants produce significant amount of antioxidants to prevent oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity. Plant and plant products are being used as a source of medicine since long. Traditional herbal medicines form an important part of healthcare system in India. The medicinal plant selected for the present study was Catharanthus roseus L., which belongs to family Apocynaceae which is commonly known as 'periwinkle' and is an important source of indole alkaloids, which are present in all plant parts. The plant is used for the treatment of diabetes, fever, malaria, throat infections, and chest complaints. It is also used for the regulation of menstrual cycles, and as a euphoriant[4]. The physiologically important and antineoplastic alkaloids namely Vincristine and Vinblastine are mainly present in the leaves whereas antihypertensive alkaloids such as ajmalicine, serpentine, and reserpine are found in the roots.[5] Vincristine and Vinblastine alkaloids are used in the treatment of various types of lymphoma and leukemia[6,7]. These Catharanthus alkaloids are also used for the treatment of both malignant and non-malignant diseases and in platelet and platelet associated disorders. In the present work, an attempt has been made to study the in vitro antioxidant activities of two varieties of Catharanthus roseus, which are distinguishable on the basis of their flower

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colors Catharanthus 'rosea' (pink colored flowers) and Catharanthus 'alba' (white colored flowers).

Materials and Methods

Reagents

All the chemicals used were of analytical grade obtained from S.D. Fine Chemicals Pvt. Ltd., Mumbai, Sigma chemical company, USA and Loba chemicals, Mumbai.

Preparation of the plant extracts

The flowers of C. rosea and C. alba were collected from the campus of Govt. College of Pharmacy, Karad and further authenticated by the Department of Botany, Science College, Karad. The air dried and powdered flowers of C. rosea and C. alba (200g) each were separately Soxhlet extracted exhaustively with (95%) ethanol. The extracts were concentrated to dryness under reduced pressure in a rotary evaporator to yield dried ethanolic extracts which were stored in a dessicator and used for further studies.

Hydroxyl radical scavenging activity

This assay was based upon the benzoic acid hydroxylation method.[8] Hydroxyl radicals were generated by direct addition of iron (II) salts to the reaction mixture containing phosphate buffer. In a screw –capped tube, 0.2ml of sodium benzoate (10mM) and 0.2ml of FeSO4.7 H2O (10mM) and EDTA (10mM) were added. Then the sample solution and a phosphate buffer (pH 7.4, 0.1M) were added to give a total volume of 1.8ml. Finally, 0.2ml of an H2O2 solution (10mM) was added. The reaction mixture was then incubated at 37^0 C for 2h. Thereafter, the fluorescence was measured at 407nm emission (Em) and excitation (Ex) at 305nm. The measurement of spectrofluorometric changes has been used to detect the damage by the hydroxyl radical.

Superoxide Radical Scavenging Activity

Superoxide radical scavenging activity of the plant extract was measured according to the method of Mc Cord and Fridovich[9], which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium. All the solutions were prepared in phosphate buffer (pH 7.8). The optical density was measured at 560nm. The percentage inhibition was calculated from the formula[10].

DPPH Radical Scavenging Activity

DPPH radical scavenging activity was measured according to the method of Braca et al.[11] An aliquot of 3ml of 0.004% DPPH solution in ethanol and 0.1ml of plant extract at various concentrations were mixed and incubated at 37˚C for 30 min. and absorbance of the test mixture was read at 517nm.The percentage of inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using the formula;

Percentage inhibition = Ao - A1/ Ao X 100

Where Ao = Absorbance of the control; A1 = Absorbance of the plant extract/ standard.

Nitric Oxide Radical Inhibition Method

Sodium nitroprusside, 0.2998 gm, was weighed accurately and dissolved in distilled water to make up the volume to 100 ml in a volumetric flask [12] (10 mM). Nitric oxide, generated from sodium nitroprusside in aqueous solution at physiological pH, interact with oxygen to produce nitrite ions, which were measured by using Griess Ilosvog Reaction.[13] The reaction mixture (6ml) containing sodium nitroprusside (10 mM, 4ml), phosphate buffer saline (1ml) and extracts (1ml) was incubated at 25 ^{0}C for 150 minutes. After incubation, 0.5ml of the reaction mixture containing nitrite was removed, 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) was mixed well and allowed to stand for 5 minutes for completing diazotization, and then 1ml of 1- Naphthylamine (5%) was added, mixed and allowed to stand for 30 minutes. A pink colored chromophore is formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solution. IC50 value is the concentration of sample required to inhibit 50% of nitric oxide radical.

Results and Discussion

The antioxidant activities of ethanolic extracts of flowers of C. rosea and C. alba were measured in different systems of assay, e.g. Hydroxyl radical scavenging assay, superoxide radical scavenging assay, DPPH assay and nitric oxide radical inhibition method. Taking 0% inhibition in the mixture without plant extract, regression equations were prepared from the concentrations of the extracts and percentage inhibitions of free radical formation. IC50 values were calculated from these regression equations. IC50 value is inversely related to the

activity. Figure 1 demonstrates the hydroxyl radical scavenging activities of the ethanolic flower extracts of C. rosea and C. alba. Hydroxyl radical is the major active oxygen centered radical formed from the reaction of various hydroperoxides with transition metal ions causing lipid peroxidation and biological damage. The scavenging of hydroxyl radical increased with increasing concentrations of the flower extracts of Periwinkle with maximum scavenging effect of 41.43 % for C. rosea and 36.68 % for C. alba at the concentration of 300 µg/ml respectively. The IC50 values of these two flower extracts were found to be 459.11 µg/ml and 473 µg/ml respectively. The results of the superoxide radical scavenging activities of the selected plant extracts are depicted in Figure 2. The maximum scavenging effect of 58.36 % and 51.33 % was observed for the flower extracts of C. rosea and C. alba respectively at a concentration of 300 µg/ml. The IC50 values for the flower extracts of C. rosea and C. alba were found to be 257.71 µg/ml and 289.67 µg/ml respectively. DPPH radical scavenging ability is widely used as an index to evaluate the antioxidant potential of medicinal plants. In the in-vitro antioxidant studies (Figure 3) the extent of DPPH radical scavenging at different concentrations of the flower extracts of Periwinkle varieties was measured. The radical scavenging effect was found to increase with increasing concentrations. The flower extracts of C. rosea and C. alba showed their maximum activity of 84.10 % and 76.67 % respectively at a concentration of 300 µg/ml .The extracts exhibited IC50 values of 185.2 µg/ml and 199 µg/ml respectively. Figure 4 shows the nitric oxide radical scavenging activities of the two plant extracts. NO is a potent diffusible free radical generated by the endothelial cells and macrophages, which is a mediator of various physiological processes. The reduction of NO radical by the flower extracts of Periwinkle varieties was found to be concentration dependent and the maximum scavenging effect was found to be 35.66% for C. rosea and 31.05 % for C. alba extracts respectively with the IC50 values of 464.5 µg/ml, and 483.7 µg/ml respectively. Figure 5 highlights the comparative representation of the IC50 values (mcg/ml) exhibited by ethanolic extracts of flowers of C. rosea and C. alba in the various in-vitro antioxidant assay systems.

Conclusion

The present study revealed that the ethanolic extracts of flowers of C. rosea and C. alba exhibited satisfactory scavenging effect in all the radical scavenging assays in a concentration dependent manner. However from this study it is concluded that the ethanolic extract of flowers of Catharanthus rosea had more antioxidant activity than Catharanthus alba, as evidenced from the various in vitro antioxidant assays.

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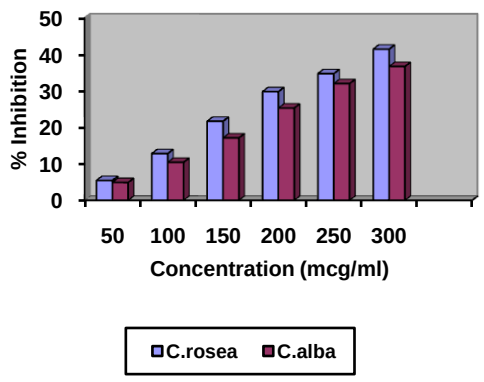

Figure 1: Hydroxyl radical scavenging potential of C. rosea and C. alba.

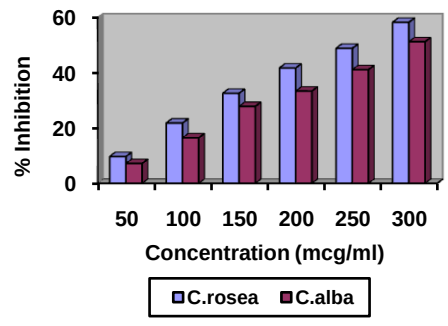

Figure 2: Superoxide radical scavenging potential of C. rosea and C. alba.

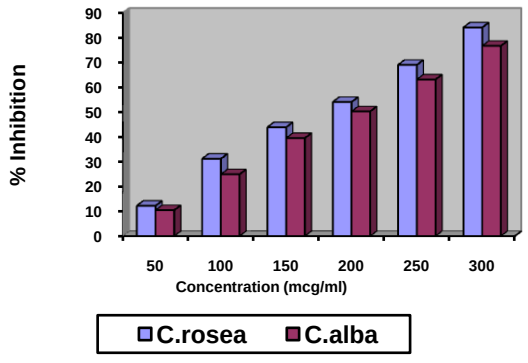

Figure 3: DPPH radical scavenging potential of C. rosea and C. alba.

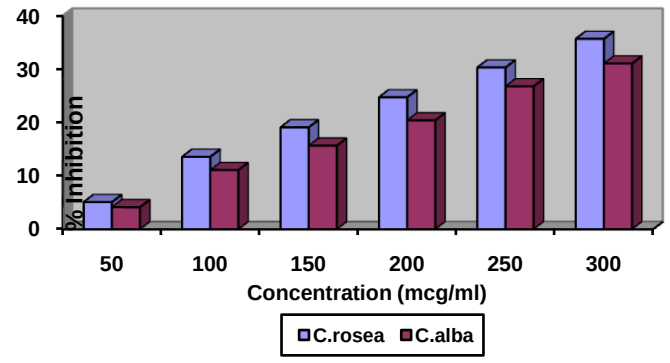

Figure 4: Nitric oxide radical scavenging potential of C. rosea and C. alba.

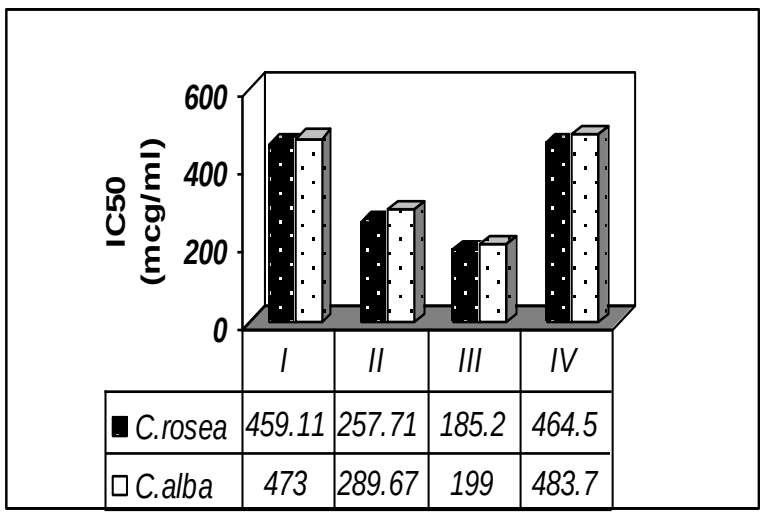

Figure 5: Comparison of IC50 values of C.rosea & C. alba extracts in Hydroxyl radical scavenging assay (I), Superoxide radical scavenging assay (II), DPPH radical scavenging assay (III) and Nitric oxide radical scavenging assay (IV).

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